Biomolecular Mechanisms Controlling Metal and Radionuclide Transformations in *Anaeromyxobacter dehalogenans*

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1867

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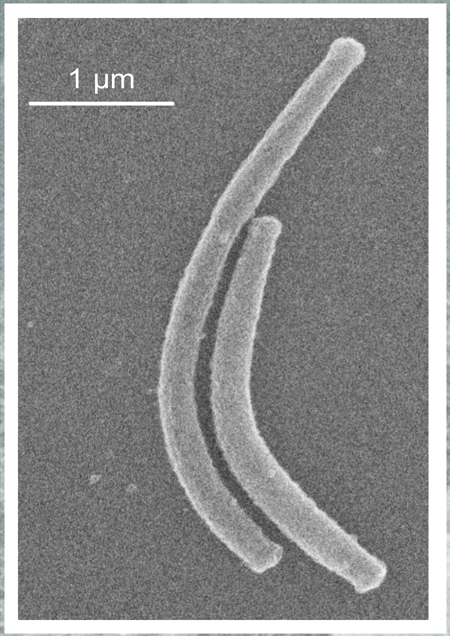
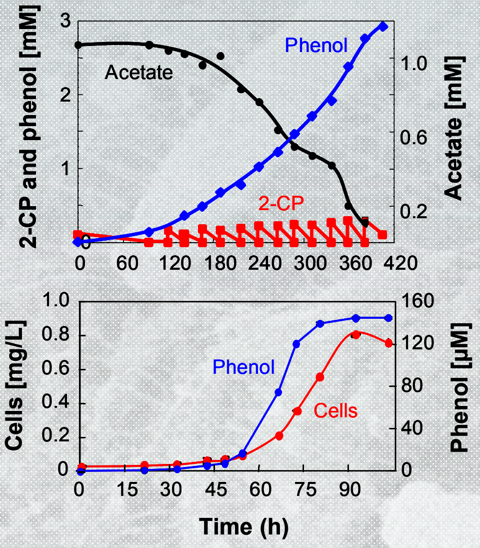
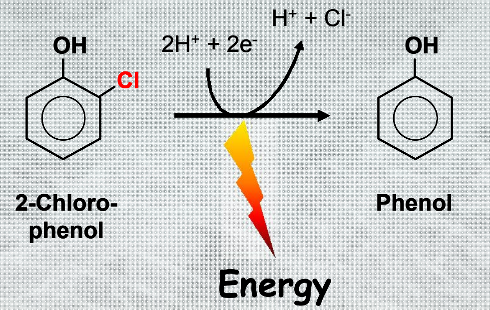
Anaeromyxobacter dehalogenans 2CP-C

- Gram-negative, facultatively anaerobic organism
- Isolated from anaerobic enrichments (tropical soil) with monochlorophenol
- Capable of reductive dechlorination (dechlororespiration) coupled to growth





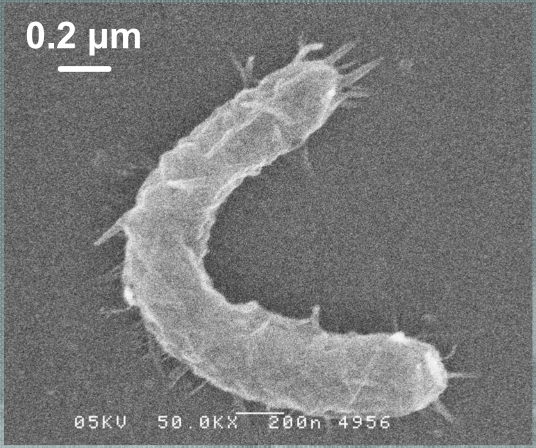


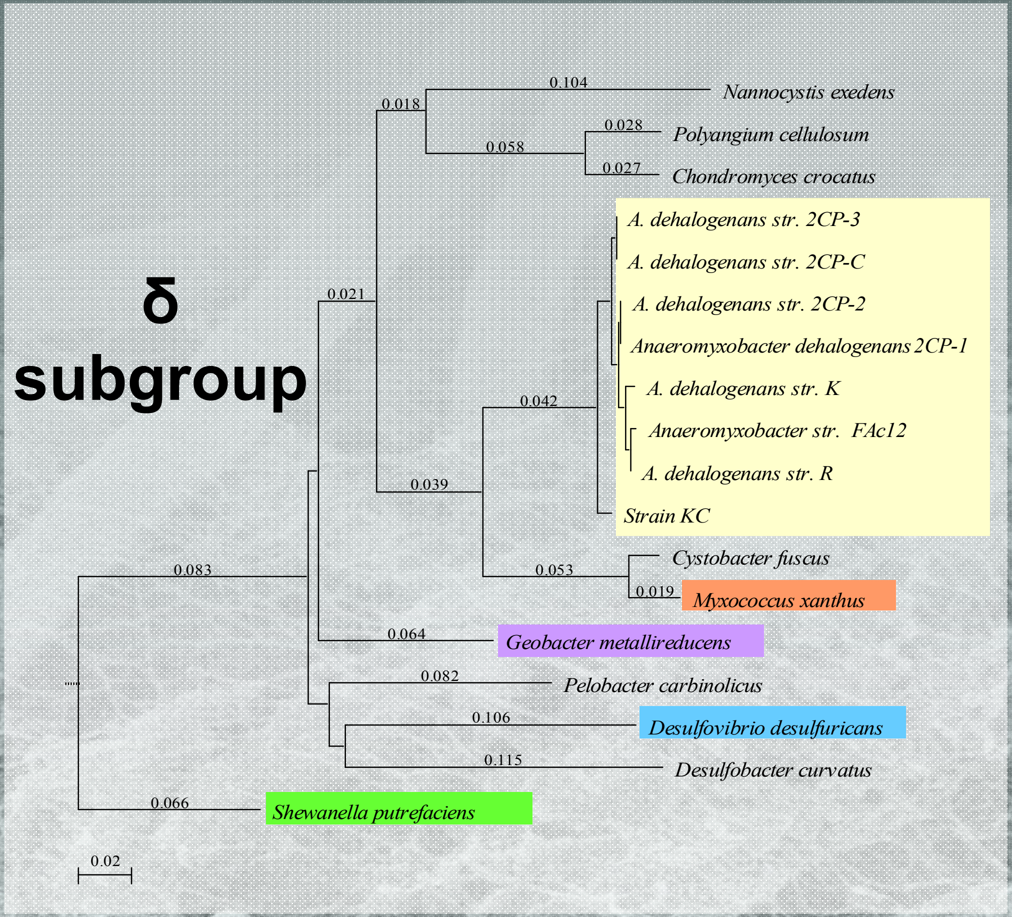
$\Delta G^{\circ\prime}$ = -157 kJ/rx with H_2

Respiratory Reductive Dechlorination
(De)Chlororespiration

Sanford et al., 2002. Appl. Environ. Microbiol., 68:893-900

Phylogeny of *Anaeromyxobacter*





Phylogenetic tree based on nearly complete 16S rRNA gene sequences

Metabolic properties of *A. dehalogenans* 2CP-C

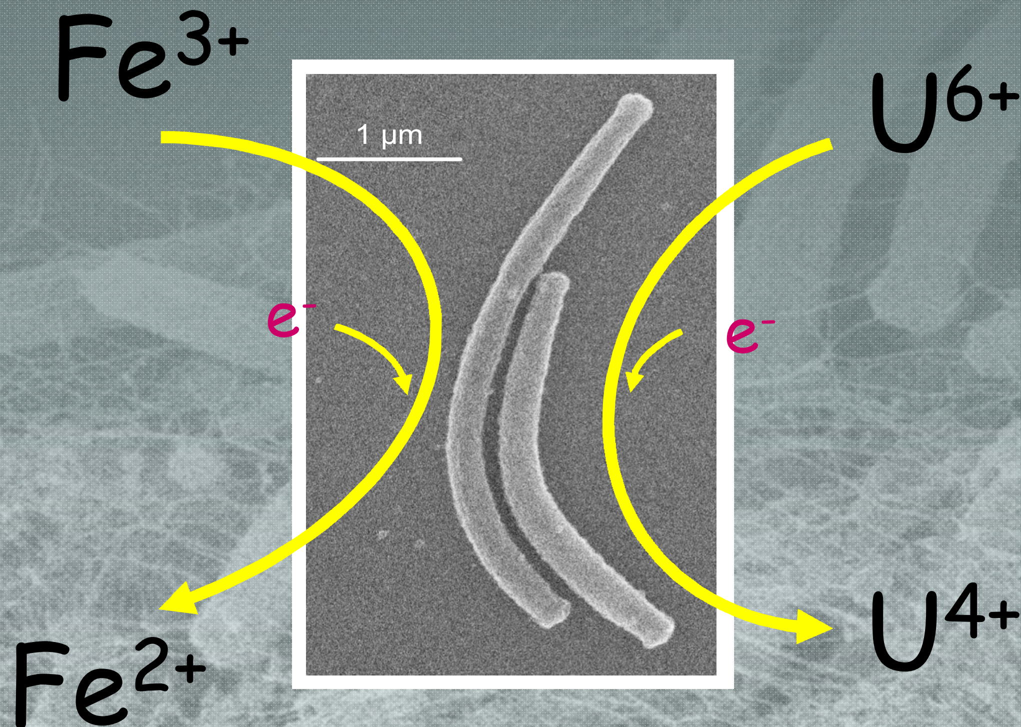
Electron Acceptors

- Oxygen
- Ortho-substituted halophenols
- Nitrate
- Nitrite
- Fumarate
- **Soluble and insoluble oxidized metal species**

Electron Donors

- Acetate
- Hydrogen
- Succinate
- Pyruvate
- Formate
- Lactate

Metal reduction *A. dehalogenans* 2CP-C

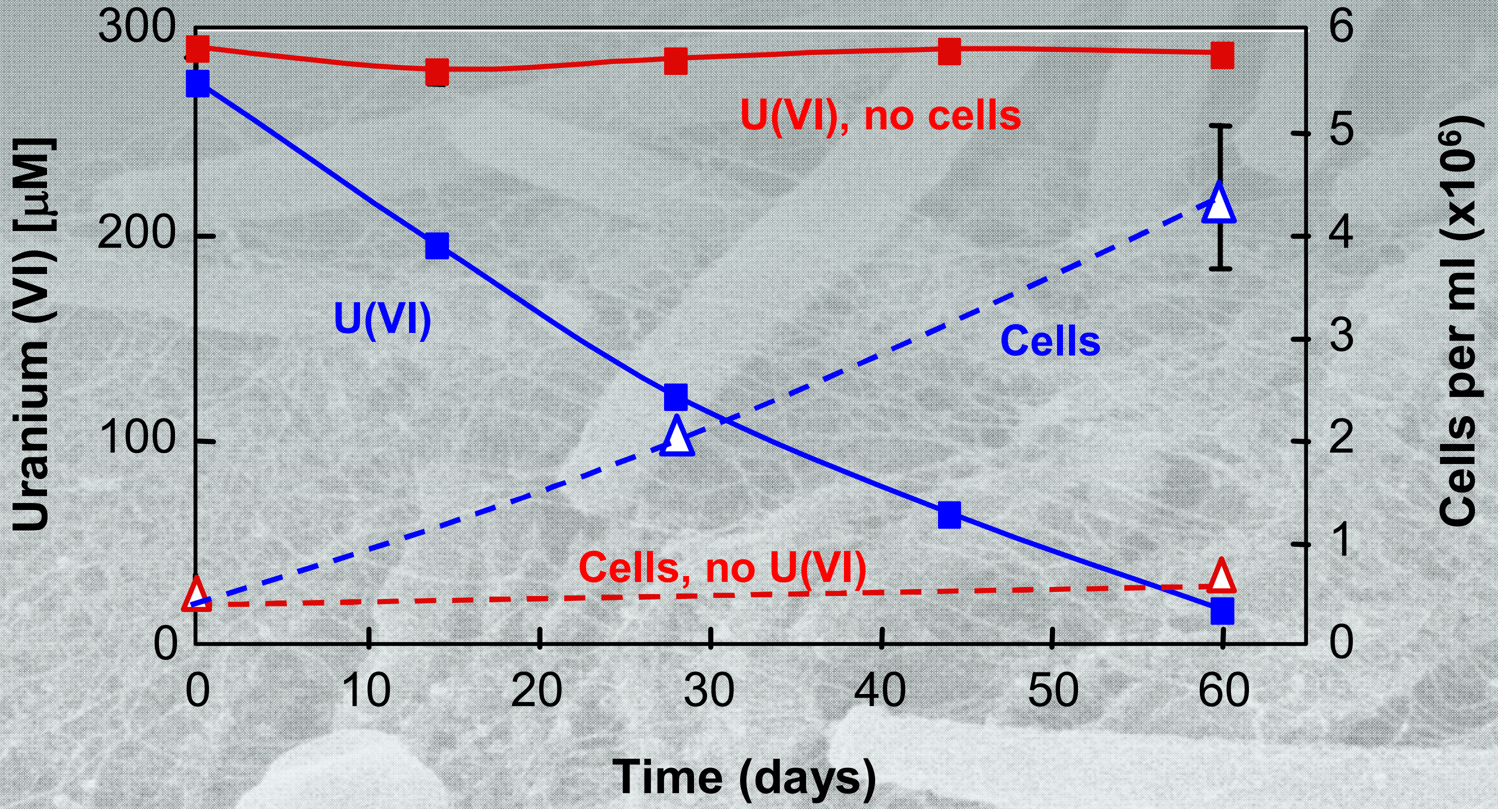


Anaeromyxobacter dehalogenans strain 2CP-C

He & Sanford, 2003. Appl. Environ. Microbiol., 69:2712-2718

A. dehalogenans 2CP-C couples U(VI) reduction to growth

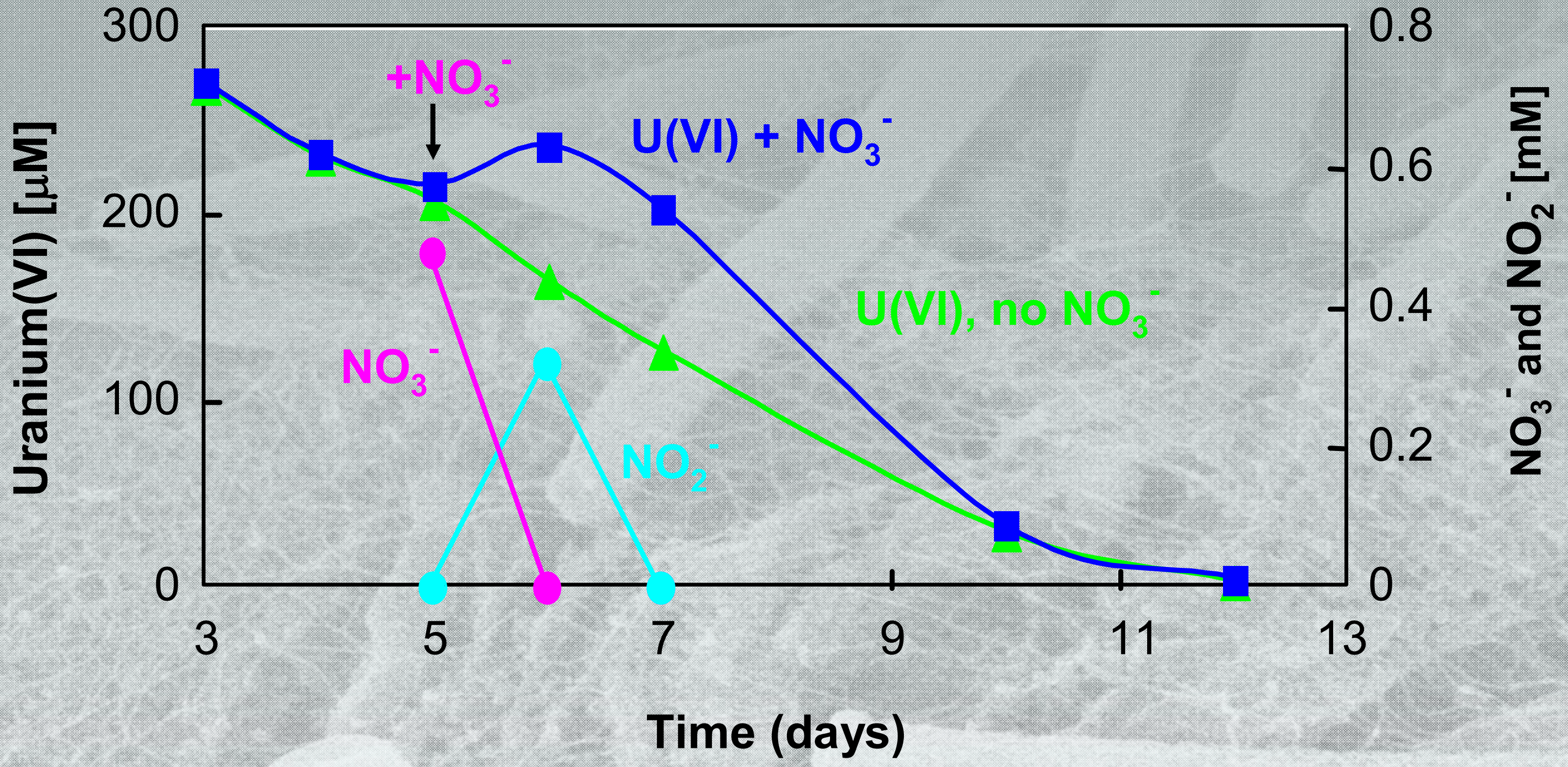
Mineral salts medium, acetate, H_2
2% (v/v) inoculum



Effect of nitrate on U(VI) reduction by *A. dehalogenans* 2CP-C

2CP-C grown with acetate/NO_3^- (0.5 mM)

U(VI) and H_2 added after NO_3^- had been consumed (day 3)



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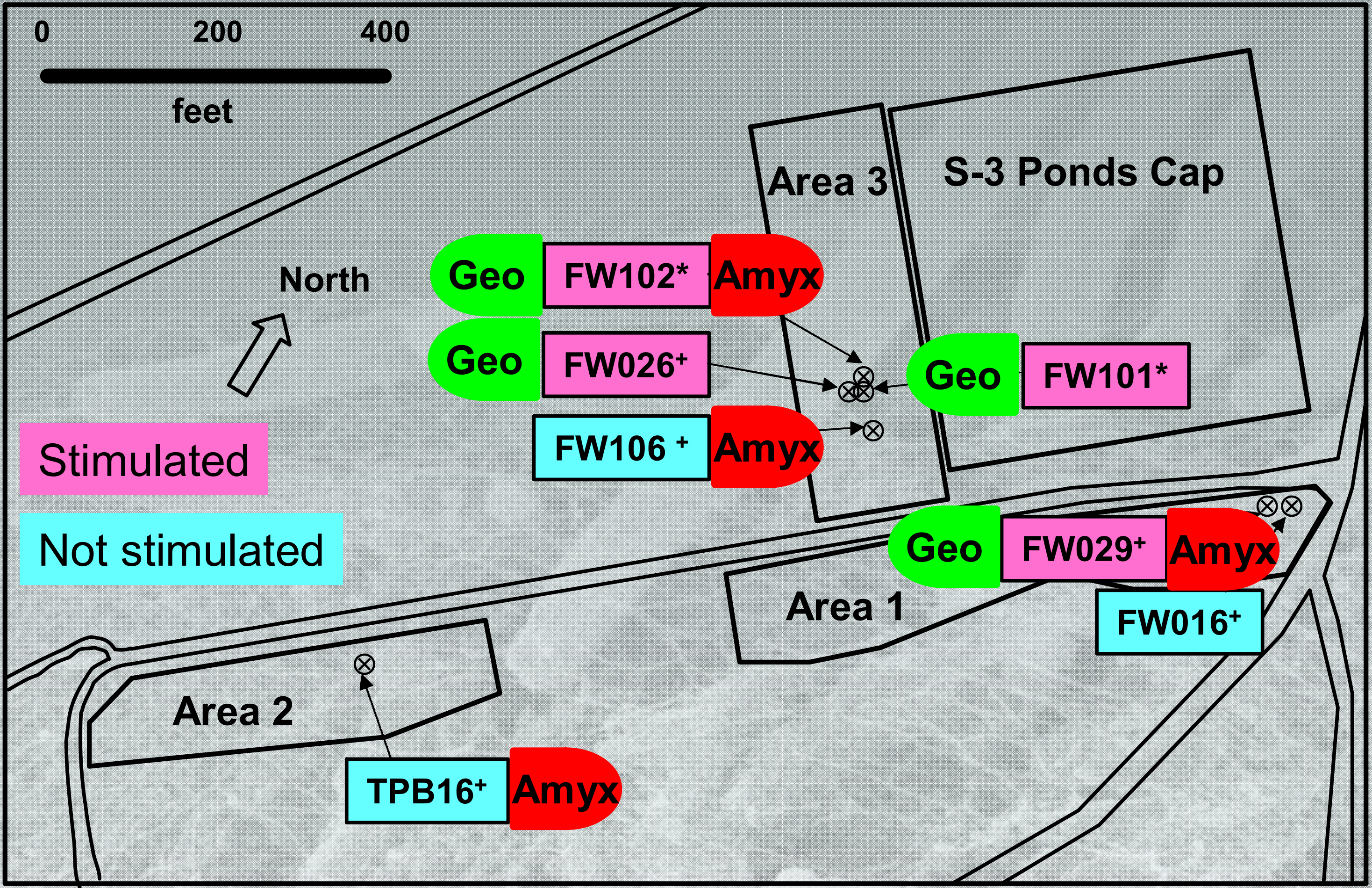


* multiport wells
+ wells

Project objectives

- Identify genes involved in metal/radionuclide reduction in *A. dehalogenans* strain 2CP-C regulated under different redox conditions

- Compare the pathways of metal/radionuclide reduction in *A. dehalogenans* 2CP-C to those found in other DMRB

- Identify the key environmental factors specific to subsurface environments that affect the expression of *A. dehalogenans* genes involved in metal/radionuclide reduction

Initial goals

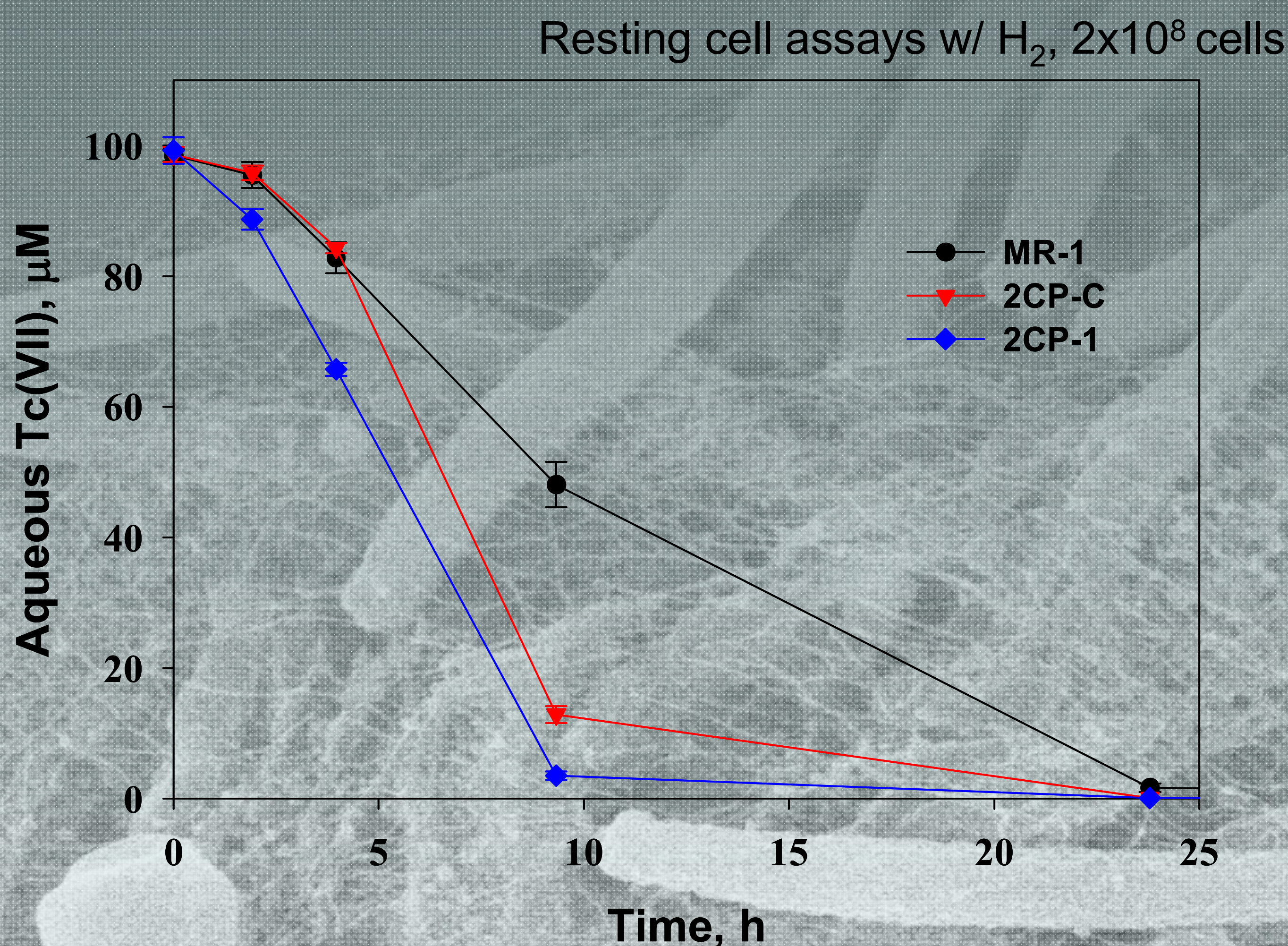
- Carry out resting cell experiments using non-growth conditions to study the kinetics of U(VI) and Tc(VII) reduction in *A. dehalogenans* strains 2CP-C and 2CP-1

- EM imaging of U(VI)-reducing cells (strains 2CP-C and 2CP-1) to study the localization of U(IV) material

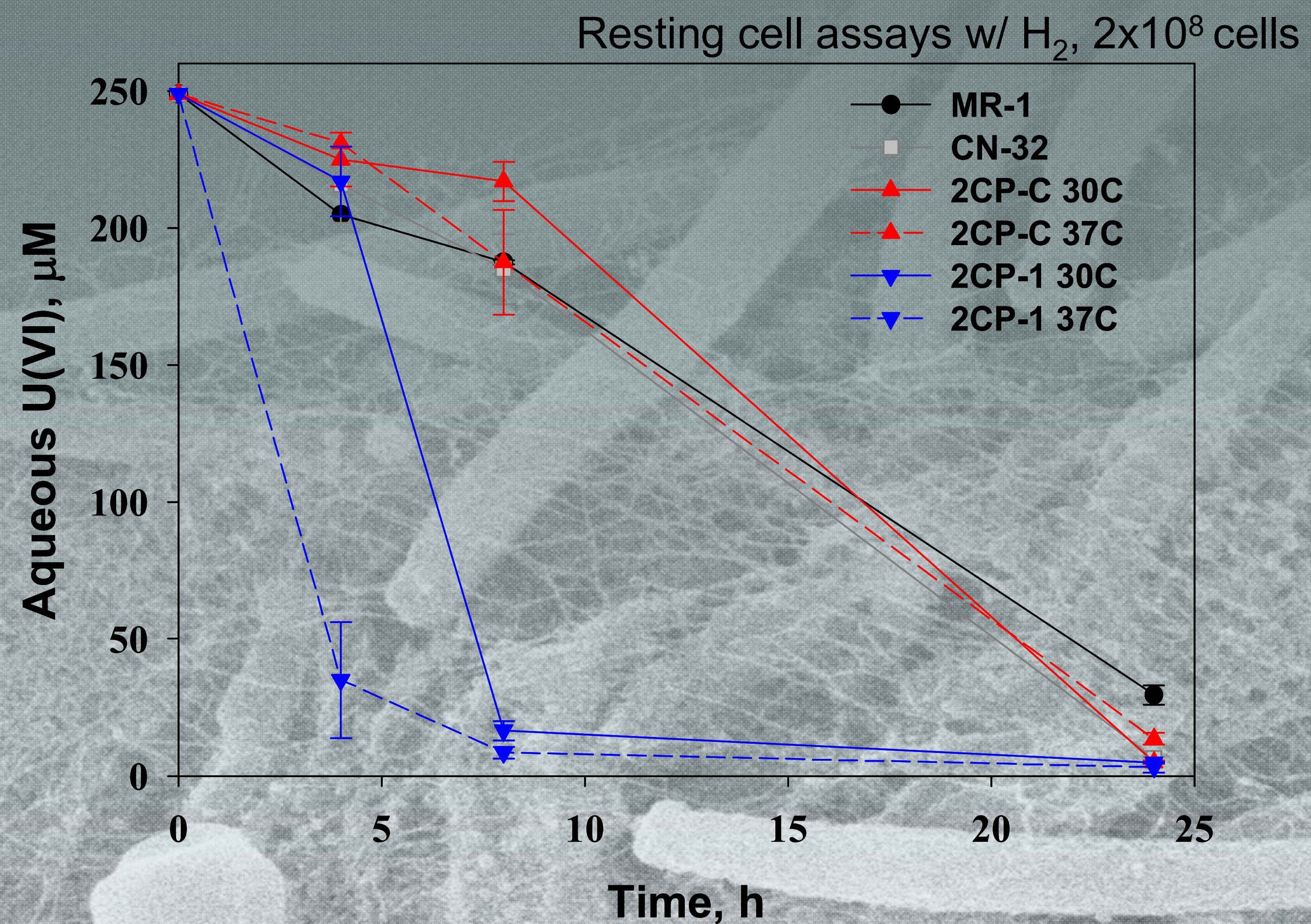
- Compare the data with previous results generated for other metal-reducing organisms (*Shewanella*)

- Identify target genes putatively involved in metal and radionuclide reduction in *Anaeromyxobacter*

Tc (VII) reduction by *A. dehalogenans*



U(VI) reduction by *A. dehalogenans*



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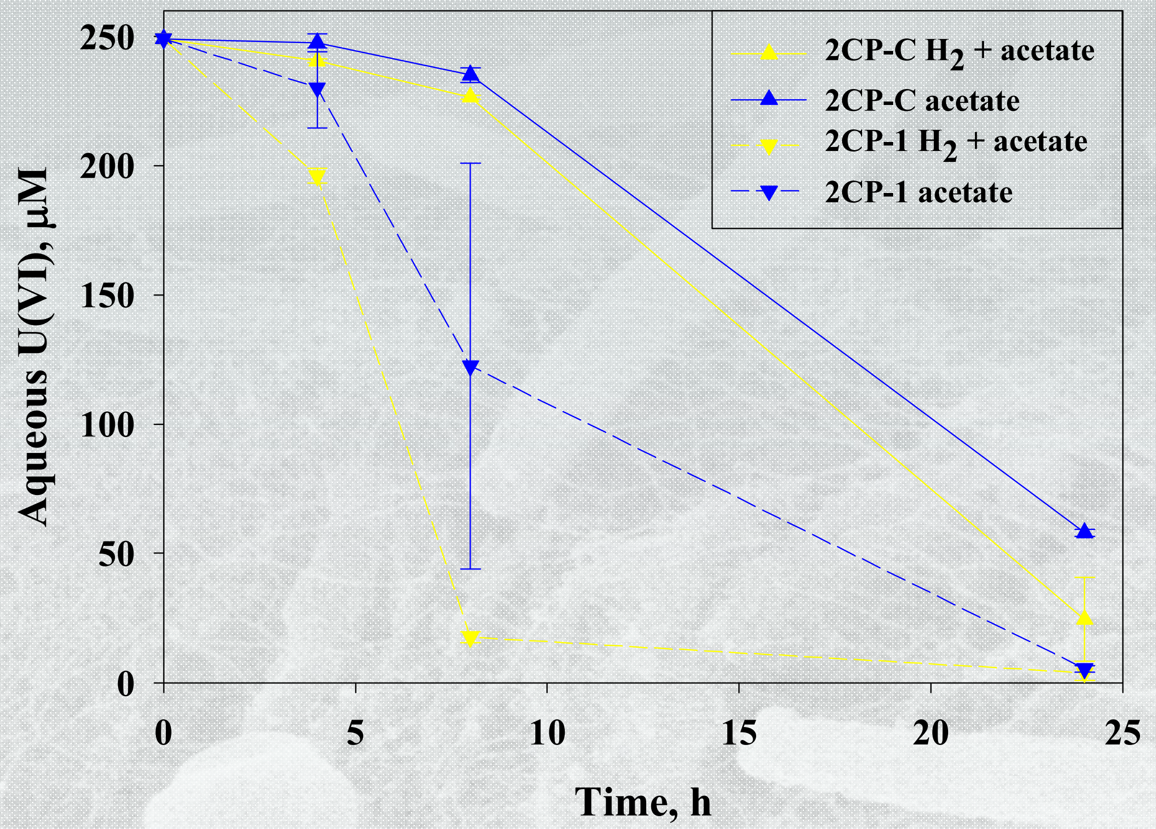
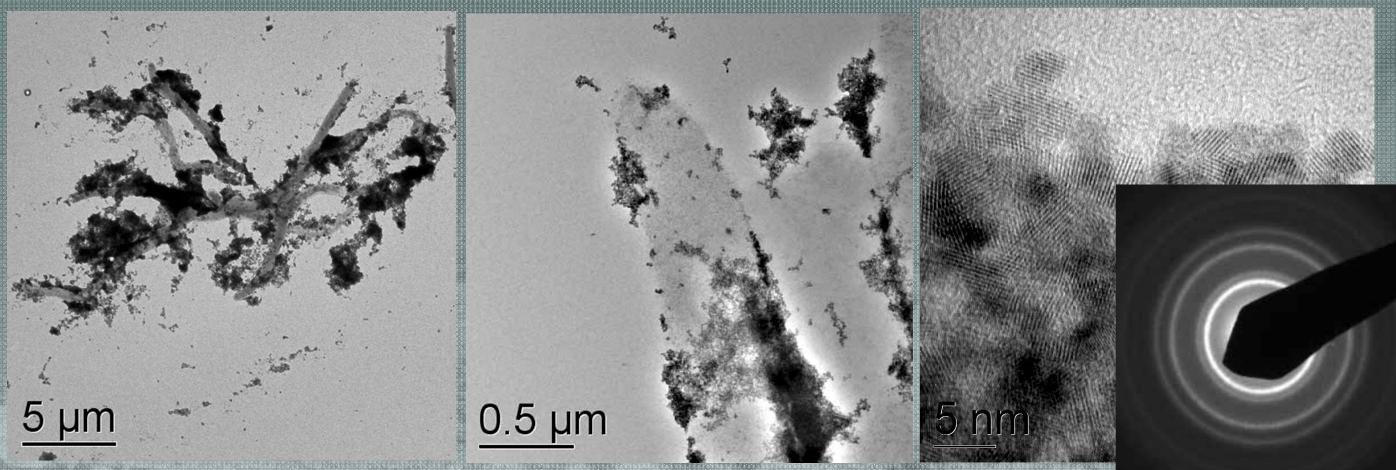
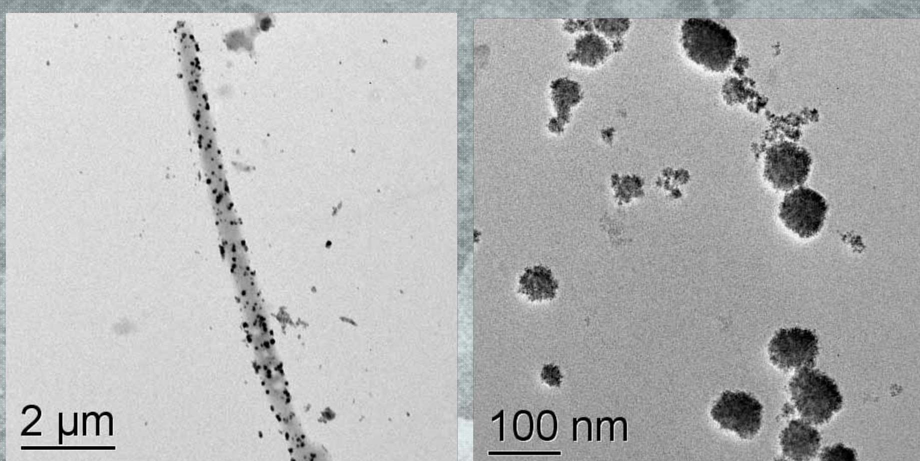
U(VI) reduction by *A. dehalogenans (acetate, 30^{o}C)*

Resting cell assays w/ H_2, 2x10^8 cells



Reduced U(IV) nanoparticle localization in *A. dehalogenans* (H_2, 30^{o}C)

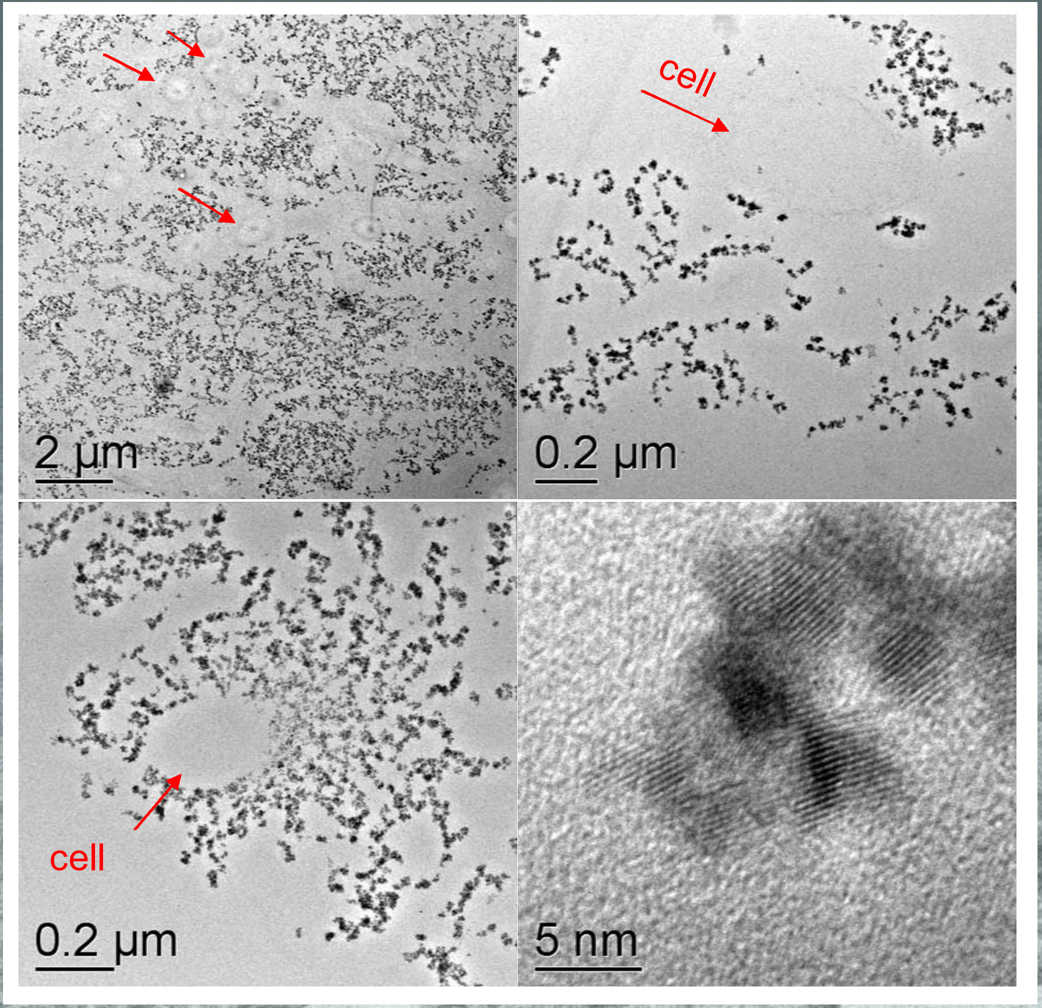




Whole mounts:

U(IV) material is present as a heavy decoration of extracellular polymers. Cells are covered with a very fine coat . Material shows an excellent match with uraninite (very similar to *Shewanella* experiments). A typical particle size is in a 5 nm range, but also with d-lines extending 10+ nm. An interesting distribution of U was on many cells – uraninite forming nodular patterns

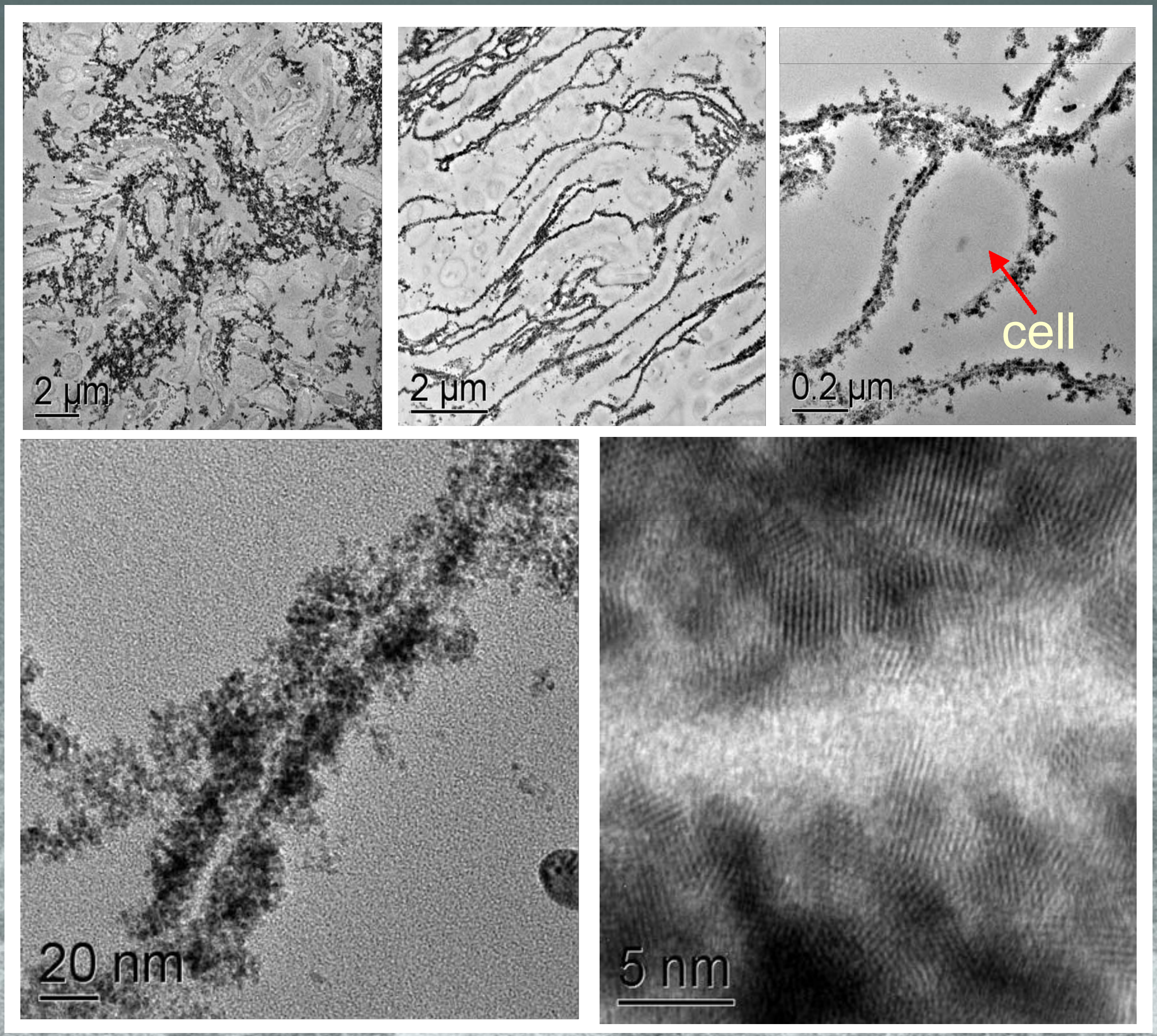
Reduced U(IV) nanoparticle localization in *A. dehalogenans* (H_2, 30^{o}C)



Thin sections:

Reduced U(IV) is bound to dispersed (amorphous) extracellular material which forms dendric-like structures. The U nanoparticles are > 5 nm in diameter. Absolutely no periplasmic deposition is found.

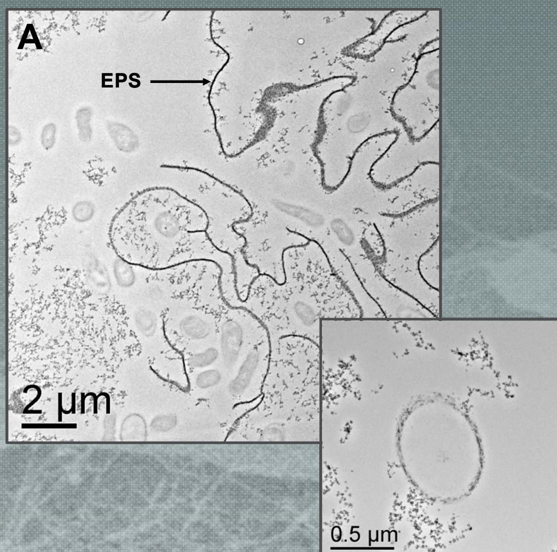
Reduced U(IV) nanoparticle localization in *A. dehalogenans (H_2, 30^{o}C)*

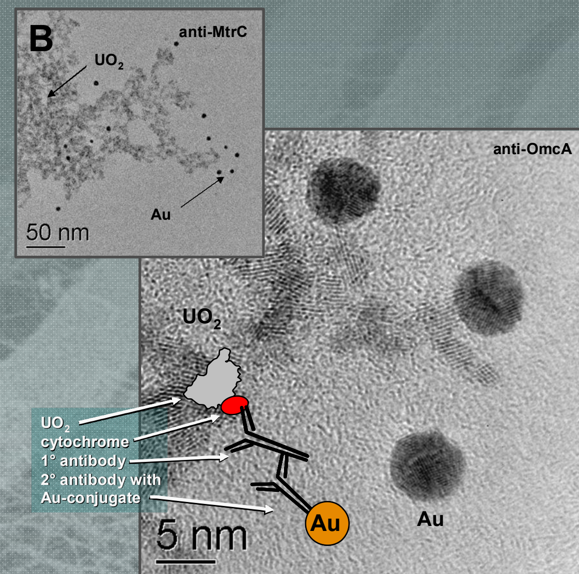


Thin sections:

U material is also bound to organized sheet-like EPS structures which are abundant throughout the sample and appear to have the same familiar morphology as known from *Shewanella* species. A thin (lipid?) core layer that is clear of UO_2, but surrounded by U nanoparticles from both sides is visible (D,E). Frequently, cells are attached to these structures (C), as if sheets are spinning out of the cell membranes, incorporating them into their network.

Are the molecular mechanisms of U(VI) reduction in *Anaeromyxobacter* similar to those in other DMRB?





In *S. oneidensis* MR-1, UO_2 nanoparticles accumulate in multiple locations : extracellular to the cells, periplasmic, and at high densities with an exopolymeric substance (EPS)

Immune-electron microscopy (EM) reveals that OM-associated, c-type decaheme cytochromes of (MtrC and OmcA) in MR-1 were primarily localized with the extracellular UO_2-EPS matrix .

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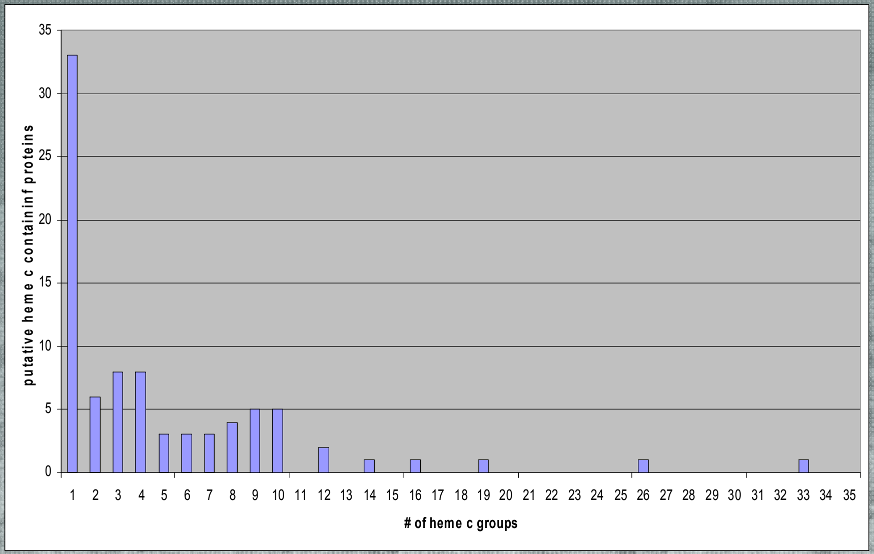
Are *c*-type cytochromes involved in radionuclide/metal reduction in *Anaeromyxobacter* species?

A glimpse of the *A. dehalogenans* 2CP-C draft genome



93 genes with CXXCH motif

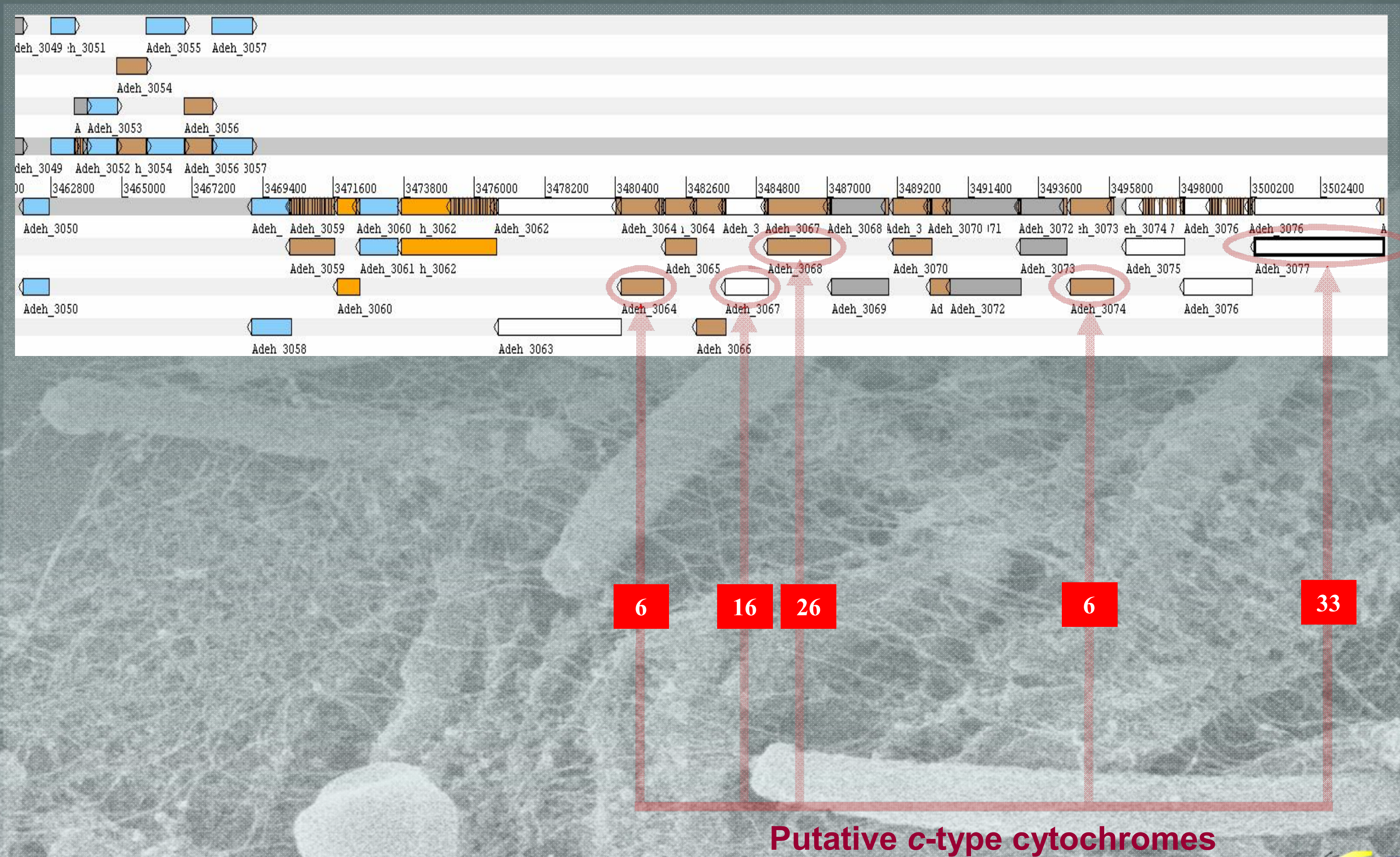
15 genes > 10 CXXCH motifs

1 with 20 CXXCH motifs

1 with 26 CXXCH motifs

1 with 33 CXXCH motifs

Organization of a 40-kb *c*-type cytochrome gene cluster in *A. dehalogenans* 2CP-1



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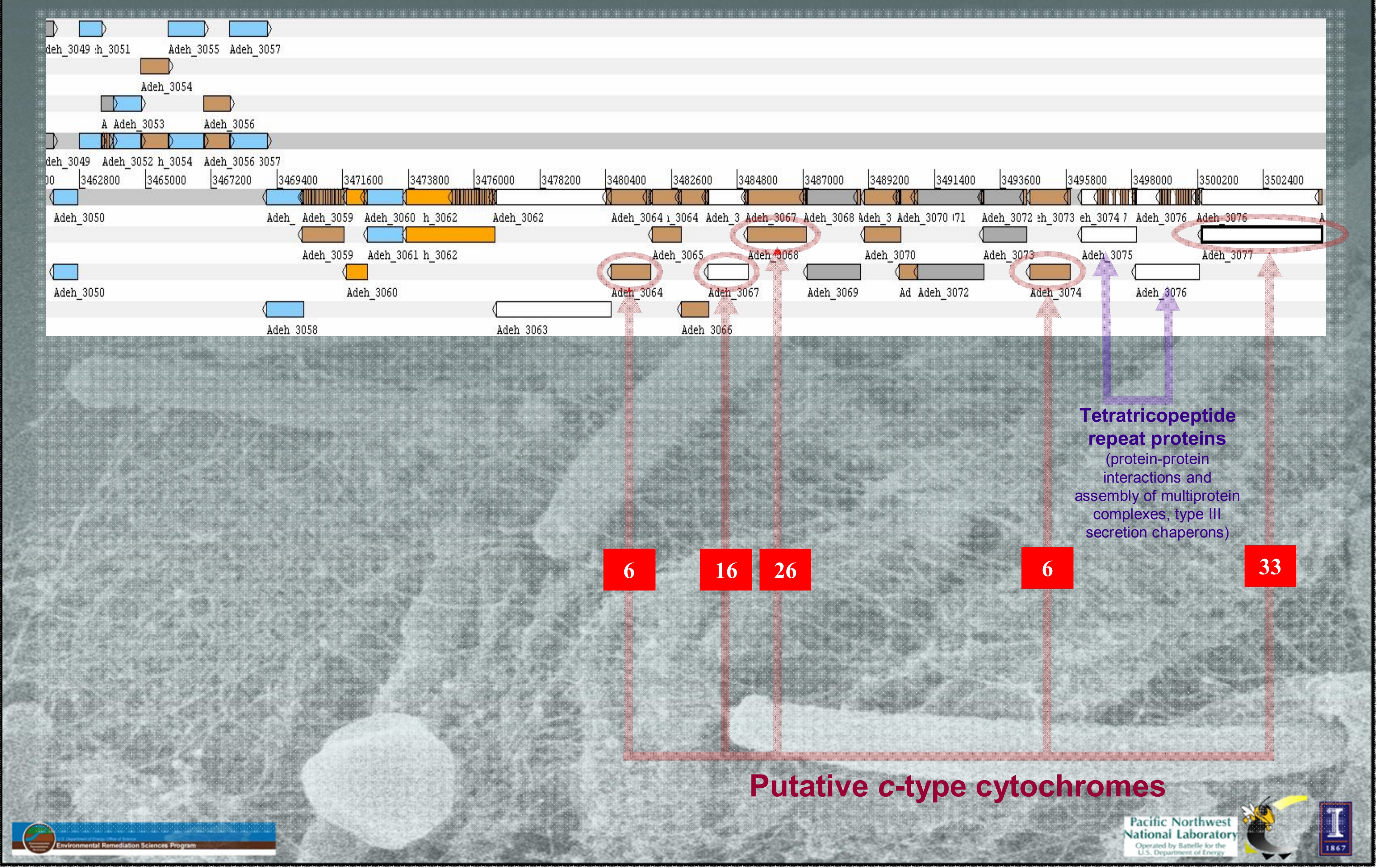
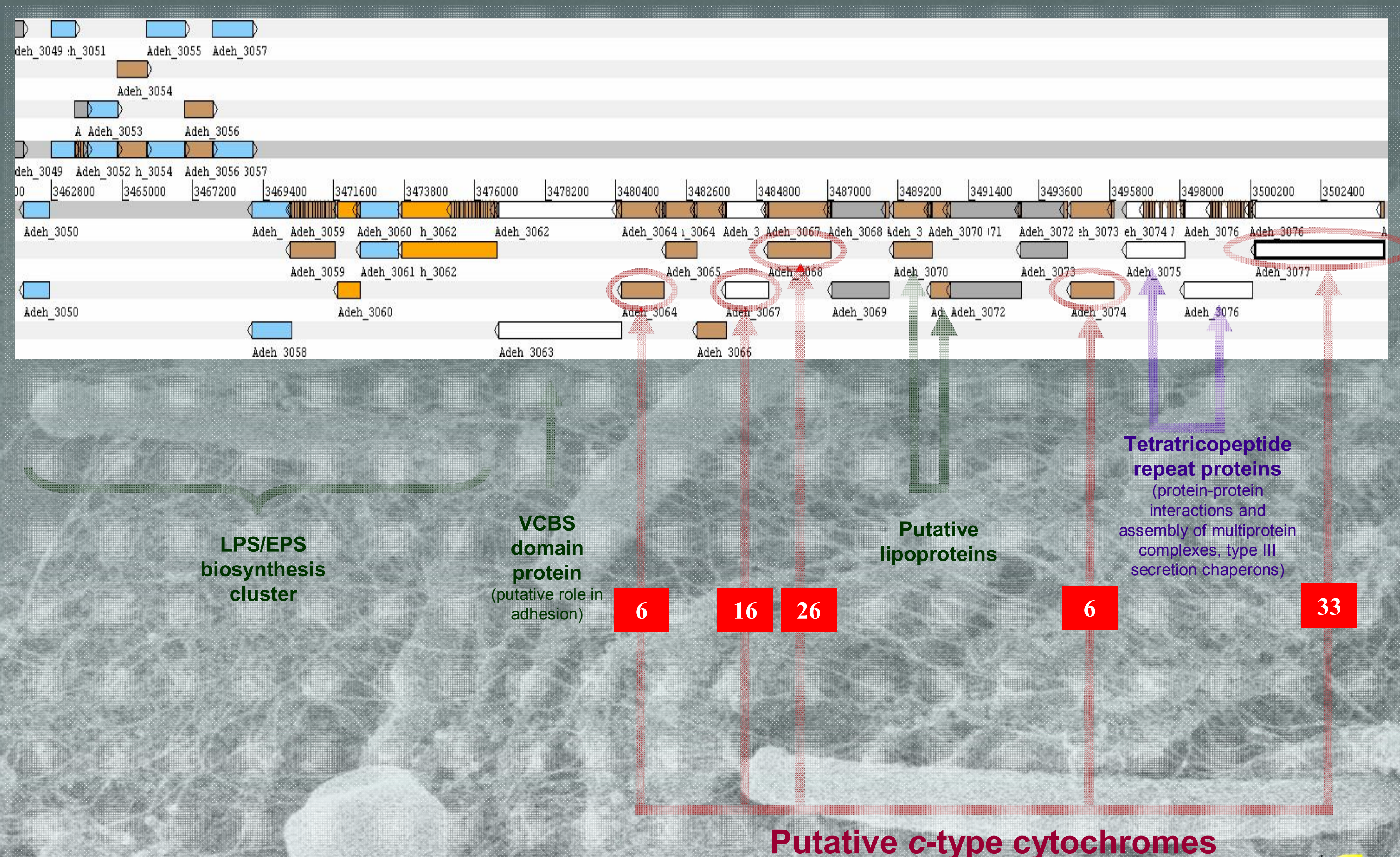
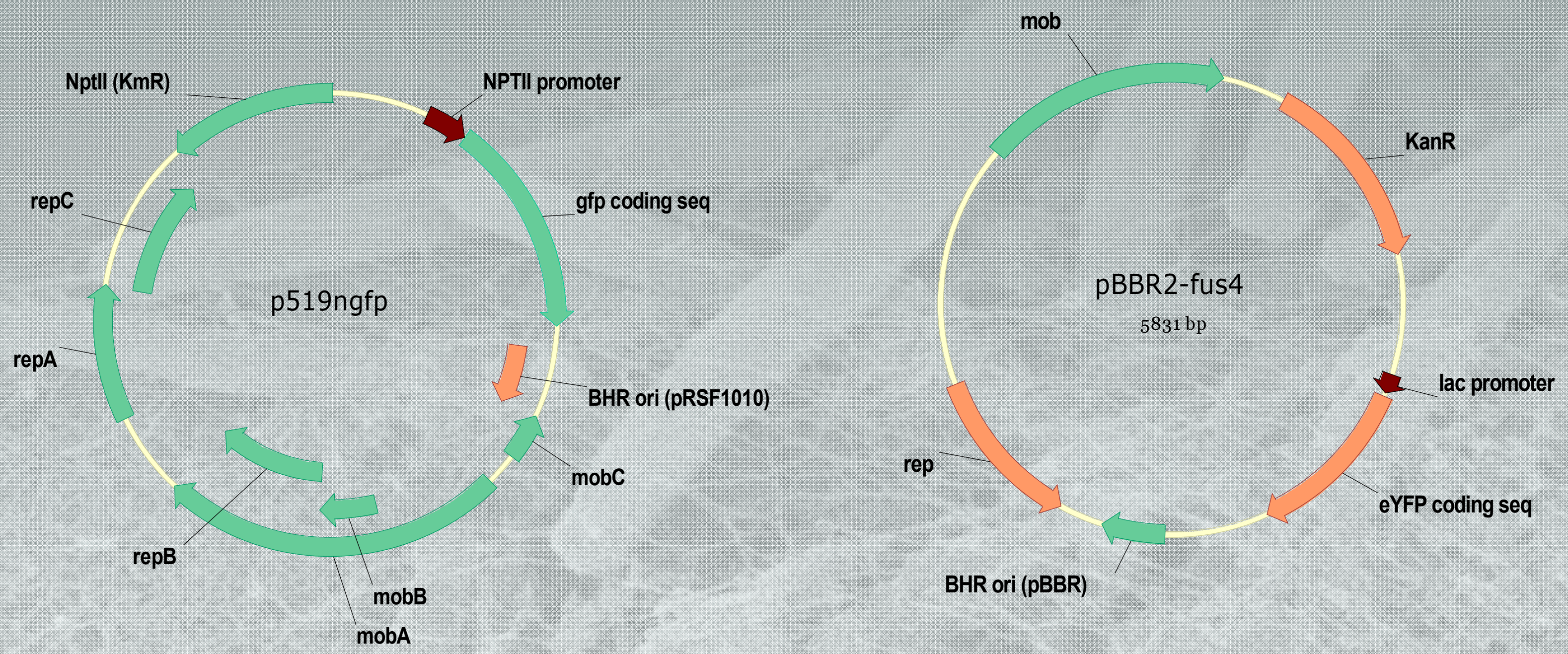
Organization of a 40-kb *c*-type cytochrome gene cluster in *A. dehalogenans* 2CP-1



Organization of a 40-kb *c*-type cytochrome gene cluster in *A. dehalogenans* 2CP-1



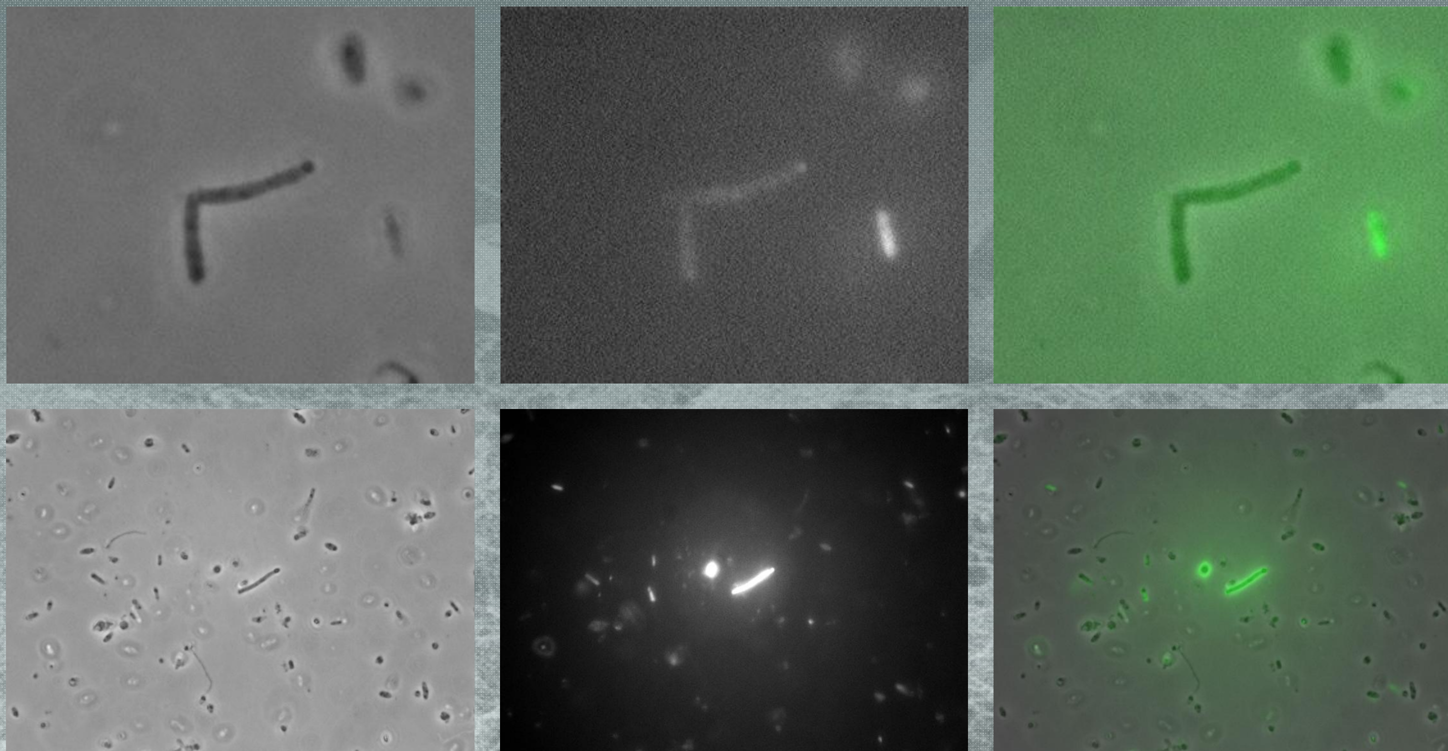
Development of a genetic system for *A. dehalogenans* 2CP-C



Plasmids conjugated into *A. dehalogenans* 2CP-C

Development of genetic system for *A. dehalogenans* 2CP-C

Light | Fluorescence | Merged



A. dehalogenans 2CP-C with the RSF1010 BHR plasmid p519ngfp (expression of GFP using constitutive _lac_ promoter)

Next steps

- Targeted mutagenesis to identify the function of the high-molecular *c*-type cytochromes found in 2CP-C

- Identification of genes essential for metal & radionuclide reduction through random mutagenesis

- Generation of whole-genome microarrays to study gene expression under different respiratory conditions

- Comparative genomic studies of different *Anaeromyxobacter* strains (2CP-C, 2CP-1, strain K, FW-109)

Related Poster Presentations

- Q. Wu *et al*., "Uranium (VI) reduction by *Anaeromyxobacter dehalogenans*", PI - F.E. Löffler

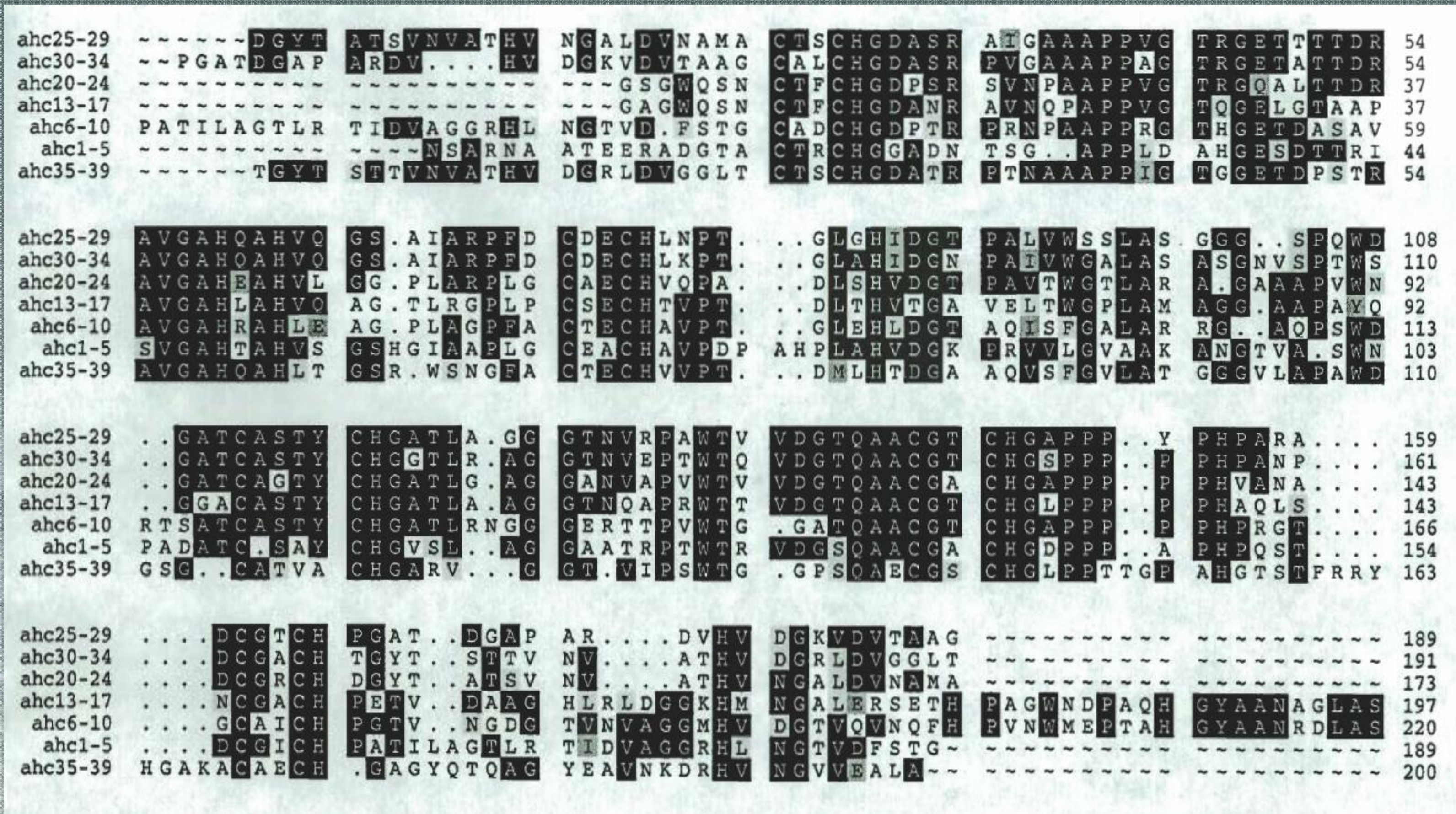
- M. Marshall *et al*., "Biomolecular mechanisms of U(IV)O_2 and Tc (IV)O2 nanoparticle formation by *Shewanella oneidensis* MR-1", PIs – J. K. Fredrickson and J.M. Zachara.

- J.R. Dale *et al*., "Mechanism of uranium and technetium reduction by metal-reducing members of the genus *Shewanella*", PI – T. J. DiChristina

Relevant characteristics of *A. dehalogenans*

- High rates of ferric iron reduction (constitutive)
- Reduce (immobilize) U(VI)
- Rapidly reduce chlorophenols to phenol
- Metabolic versatility (e^- acceptor and e^- donor)
- Genome analysis suggests abundance of *c*-type cytochromes
- *Anaeromyxobacter* 16S rRNA gene sequences retrieved from high NO_3^-, low pH, radionuclide-contaminated FRC site

Domain alignment of *Adeh_3077*



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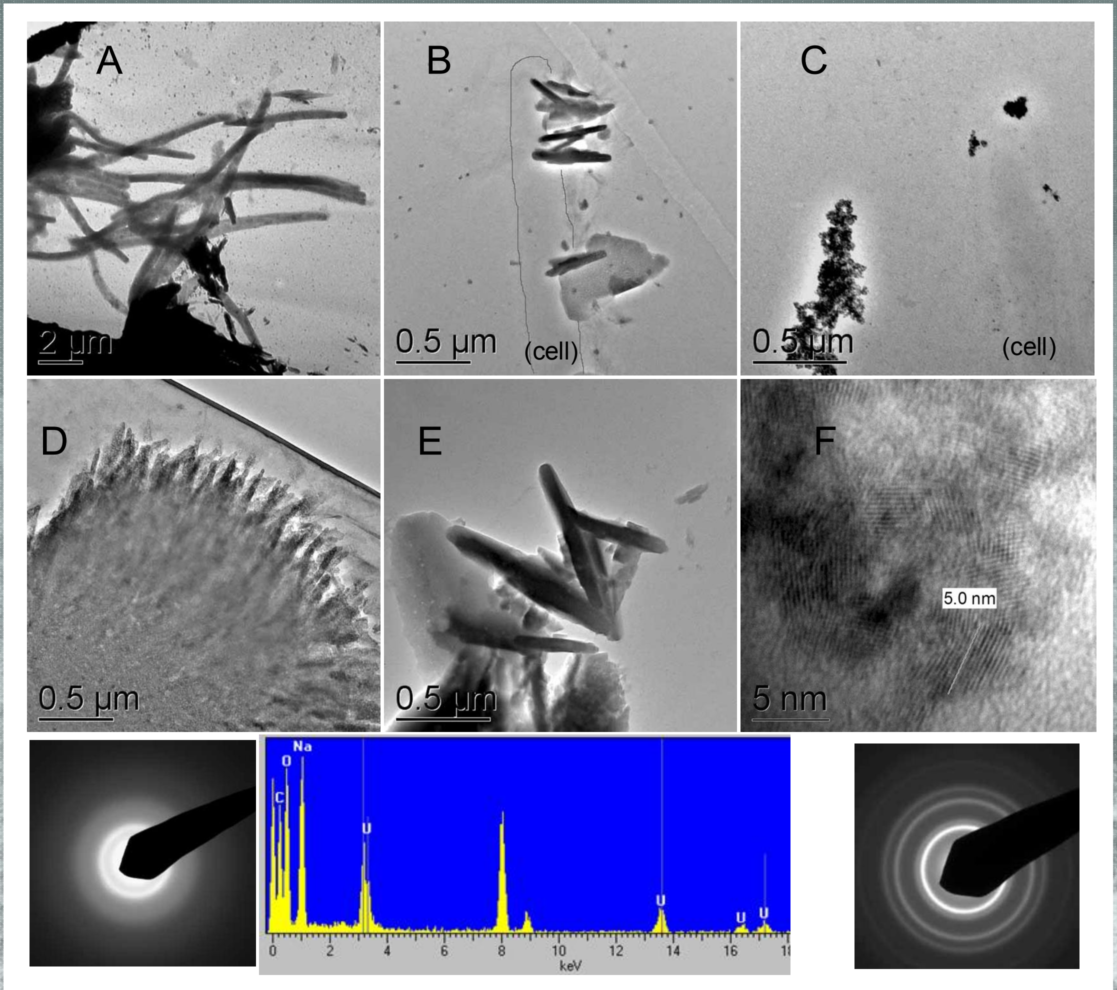
Reduced U(IV) nanoparticle localization in *A. dehalohegans (acetate, 30^{o}C)*



Whole mounts:

The sample with acetate has a large Na signal on top of U/O. Distinctive needle-like large crystals were found throughout the sample. Areas of uraninite with very little Na signal was found also (C). Cells were mostly clean (A), and didn't show much of exPS or fine material on their surface.